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On the experimental production of lack of carbohydrates, and on the carbohydrate metabolism of the central nervous system.

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To gain further insight into the genesis of the hypoglycaemic symptoms and other problems of carbohydrate metabolism it appeared advisable to dispose of various methods of depriving the organism of its stores of carbohydrates. In various former publications from the Berne Physiological Institute, mention has been made of methods suitable for this purpose. We have now worked out such a method that one is able experimentally to deprive the organism almost completely of its store of carbohydrates. The method, as applied to rats, consists in the following procedure: The rats are fed for a few days with Witte's pepton, then with thyroid gland preparations, and finally phlorhizin is injected according to Coolen's methods, and the animals are then worked in a treadmill. The treatment with Witte's pepton and with thyroid gland preparations must be carefully regulated according to the condition of the animals; they are especially sensitive to feeding with Witte's pepton. With this method we succeeded in lowering the carbohydrates of the liver 96 per cent, the carbohydrates of the muscle 85 per cent, and the blood sugar to the same low value as in the hypoglycaemic state after insulin.

We were particularly interested in the carbohydrate content of the brain. We found per gram of brain an average amount of 1.23 mg. carbohydrate of which 0.75 mg. is glycogen. This so-called glycogen is the product of analysis according to the method of Pflüger.

As a result of our experimental proceedings the carbohydrates, especially the content of glycogen, had been diminished by something like 90 per cent, the glycogen content of the brain had not diminished more than about 20 per cent. Also after insulin, where we obtained in the case of the liver, the muscles and the blood, the same results as were attained by the method described

above, the glycogen content of the brain was only slightly decreased. The amount of this diminution seemed to depend upon whether or not the animals showed symptoms of convulsions. Therefore we injected strychnine. Like insulin this drug only causes a type of convulsions which is not very marked. Although the decrease in glycogen content of the brain in these cases was more pronounced, the diminution of the carbohydrates of the tissues is at the same time very much less than in the former experiments.

We then turned to rabbits as the effect of insulin upon these animals is far more pronounced. In normal rabbits, on the average, the sum total of the carbohydrates of the brain was 0.79 mg. per gram of brain, of which 0.39 mg. was glycogen. Injections of insulin, when they led to the well known strong convulsions, diminished the glycogen storage of the brain over 80 per cent. While if insulin was given without leading to convulsions, we observed a small but distinct increase of all the carbohydrates of the brain, a fact not without interest, but which is not to be discussed in this preliminary communication. The direction in which our experiments point seems to be clear: Convulsions which are due either wholly or partially to excitations of the central nervous system are capable of diminishing the carbohydrate store of the central nervous system. To prove this, we injected picrotoxin into the rabbit. This produces, by central stimulation, powerful convulsions of a distinctly different type to those occurring after insulin. There is also another difference: The convulsions are accompanied by hyperglycaemia. But what is most important, the glycogen of the brain is decreased 80 per cent, the greatest decrease we ever met with.

Before discussing the bearing of our results, a word has to be said about the chemical nature of the carbohydrates of the brain. As we had employed Pflüger's method of estimation of glycogen we spoke of glycogen in the brain as the precursor of the sugar, which ultimately was estimated. The possibility had to be taken into consideration that instead of glycogen one of the cerebrons served as the source of the sugar. This we actually found. There appeared also to be some physical-chemical difference in the properties of glycogen of the brain prepared by Pflüger's method in comparison with those of glycogen derived from the liver or from the muscle. We therefore tried another method recently

published by Rona and van Eweyck.[1] This procedure does not employ concentrated solutions of caustic potash. We altered it slightly for quantitative purposes, the chief modification being the preliminary treatment with a 3 per cent solution of sodium fluorid, which replaces somewhat in its dissolving capacity the quality of concentrated caustic alkali solutions. The substance prepared by this method from the brains of rabbits gave the characteristic reaction of glycogen, and we therefore believe to have proved the presence of glycogen in the brain. As far as our present studies go, it appears, that not all of that which according to the Pflüger method is estimated as glycogen is in reality glycogen, for the new method gives a smaller amount.

We were able, using the new method, to compare the brain of a patient who died in tetanic convulsions with that of another not suffering from a disease accompanied by a restless state of the muscles. The former brain was devoid of glycogen, while the latter showed approximately the same amount we had found in the brain of rabbits.

The principal result of our studies is the definite proof of a carbohydrate metabolism in the brain, *i. e.,* of a metabolism which allows of carbohydrate storage in the central nervous system. This carbohydrate storage is most resistant, and is, in its resistance, only surpassed by that of the carbohydrates in the heart. The stored carbohydrates of the brain are drawn into metabolism by conditions which favour a state of exaggerated excitability of the central nervous system.

[1] Rona and van Eweyck, *Biochem. Z.*, 1924, clxix, 174.